Translation

PCT/ES2003/000547

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 00.077	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/ES2003/000547	International filing date (*day/month/year*) 27 October 2003 (27.10.2003)	Priority date (*day/month/year*)
International Patent Classification (IPC) or national classification and IPC C12Q 1/68		
Applicant LABORATORIOS DR.F. ECHEVARNE, ANALISIS, S.A.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

3. This report is also accompanied by ANNEXES, comprising:

   a. ☐ *(sent to the applicant and to the International Bureau)* a total of _____ sheets, as follows:

      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).

      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

- ☒ Box No. I Basis of the report
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15 April 2005 (15.04.2005)	13 January 2006 (13.01.2006)
Name and mailing address of the IPEA/ES Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/ES2003/000547

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☒ This report is based on translations from the original language into the following language _____, which is language of a translation furnished for the purpose of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☒ The international application as originally filed/furnished

☒ the description:

pages 1-12, as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages 13-15, as originally filed/furnished

pages* _____, as amended (together with any statement) under Article 19

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the drawings:

pages 1/7-7/7, as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5-11	**YES**
	Claims	1-4, 12-15	**NO**
Inventive step (IS)	Claims	5-11	**YES**
	Claims	1-4, 12-15	**NO**
Industrial applicability (IA)	Claims	1-15	**YES**
	Claims		**NO**

2. Citations and explanations

Reference is made to the following documents:

D01: BELLIC C. *et alia*, "A molecular genetic approach for forensic animal species identification", Forensic Science International, Vol. 134, No. 134, pages 99-108, 8 July 2003

D02: LOCKLEY K. *et alia*, "Intron variability in an actin gene can be used to discriminate between chicken and turkey DNA", Meat Science, Vol. 61, pages 163-168, 2002

D03: SADAYO NAKAJIMA-IIJIMA *et alia*, "Molecular structure of the human cytoplasmic B-actin gene: Interspecies homology of sequences in the introns", Proc. Natl. Acad. Sci., Vol. 82, No. 18, pages 6133-6137, 1985

D04: KOCHER T D *et alia*, "Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers", Proc. Nat. Acad. Sci., Vol. 86, No. 16, pages 6196-6200, 1989

D05: RODRIGUEZ MIGUEL A. *et alia*, "Qualitative PCR for the detection of chicken and pork adulteration in goose and mule duck foie gras", Journal of the Science of Food and Agriculture, Vol. 83, No. 11, pages 1176-1181, September 1989

The present invention relates to methods for identifying a plurality of biological species in a single sample by PCR amplification of certain gene segments.

The applicant has devised a method which uses a composition of primers which amplify DNA fragments which differ in different biological species. The DNA sequences obtained from each of the fragments are used to search a digital data bank containing the region sequences which can be amplified by the primers selected according to the invention for various biological species.

The subject matter of claims 1-4 and 12-15 relates to a method for identifying biological species, the essential features of the method being:

1. extraction of DNA from the sample;
2. PCR amplification of beta actin gene segments.
3. identification of the amplified segment by size comparison with a reference sample, and/or DNA sequencing and comparison of the resulting sequence with the specific sequence of the species or subspecies recorded in the data bank.

While taking into consideration the applicant's response to the IPEA written opinion, the examiner continues to consider that said claims, in their present form, lack novelty. Novelty implies that the essential technical features as claimed must not have been previously disclosed in a document whose teachings would lead to a method similar to the claimed method. The applicant argues in his response that, according to D1, the beta actin gene is not useful as a tool for identifying the specific species which are listed in table 2. Nevertheless, this relates to inventive step, rather than to novelty.

What does not appear to be novel is the above-mentioned method as per claim 1 and its dependent claims. Method claims including the primers used to amplify specific regions could be novel over the cited prior art.

When assessing inventive step, D2 would be considered the prior art closest to the invention, since it describes a DNA analysis method for discriminating, by single-step PCR, the origin of a sample (chicken or turkey), and the use of primers designed depending on the intron variability of the cardiac alpha actin gene to generate products having a size which is specific to each species. Consequently, the purpose of the invention (or its object according to the applicant) clearly coincides with that of D2 and hence the choice of this document as the closest prior art is correct.

The difference between D2 and the application, in terms of essential technical features, is that the segments to be amplified correspond to beta actin and are gene segments of different gene regions from DNA sequences with high evolutionary interspecies conservation in different species and subspecies (claims 3, 6).

The present invention can be considered to address the problem of providing an alternative gene besides the already existing genes for use in devising a process for taxonomically identifying a biologically heterogeneous sample of unknown composition.

The solution to this problem is a method which amplifies gene segments from different regions of the beta actin gene from DNA sequences with high evolutionary interspecies conservation.

D3 discloses the complete sequence of the human beta actin gene and analyses the homology of interspecies intron sequences. That document shows that the beta actin exon sequences show high interspecies conservation (human vs. rat show 90% homology, human vs. chicken show more than 85% homology; page 6137, column 2, paragraph 5). It also states that the encoding regions of the contiguous exon/intron positions are identical in the genes of the various analysed species but that, nevertheless, the intron sizes differ. As relates to this difference, D3 points out that "introns show homologies ranging from 40 to 60%, except for intron III, which shows 73% homology in humans and rats". However, this intron is one of the most different in humans and chicken (39% homology).

Consequently, a person skilled in the art aware of the features of the beta actin gene (a gene with high interspecies conservation in its encoding sequences and high variability in its intronic sequences, depending on the species) would be inclined to replace it by the alpha actin gene used in D2, when devising a PCR method for discriminating the origin of a sample (from a plurality of species). In other words, the choice of the beta actin gene is an obvious choice, in view of the prior art.

However, if the claims were drafted in a clear and adequate manner, a method incorporating specific primers which amplify specific DNA regions which, because of their special interspecies homology features, would permit the DNA from different species to be identified in a heterogeneous sample, would be inventive since the choice of the specific regions to be amplified would not be an obvious choice because of interspecies differences (example of intron III).

Claims 1-15 are industrially applicable and meet the requirement of PCT Article 33(1) and 33(4).